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West Nile viral infection of equids

J. Angenvoort^a, A.C. Brault^b, R.A. Bowen^c, and M.H. Groschup^{a,*}

^aFriedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, Südufer 10, 17493 Greifswald-Insel Riems, Germany

^bCenter of Disease Control, Division of Vector Borne Diseases, NCEZID/DVBD/ADB/VA, Fort Collins, CO, United States

^cColorado State University, Department of Biomedical Sciences, Fort Collins, CO, United States

Abstract

West Nile virus (WNV) is a flavivirus transmitted between certain species of birds and mosquito vectors. Tangential infections of equids and subsequent equine epizootics have occurred historically. Although the attack rate has been estimated to be below 10%, mortality rates can approach 50% in horses that present clinical disease. Symptoms are most commonly presenting in the form of encephalitis with ataxia as well as limb weakness, recumbency and muscle fasciculation. The most effective strategy for prevention of equine disease is proper vaccination with one of the numerous commercially available vaccines available in North America or the European Union. Recently, WNV has been increasingly associated with equine epizootics resulting from novel non-lineage-1a viruses in expanding geographic areas. However, specific experimental data on the virulence of these novel virus strains is lacking and questions remain as to the etiology of the expanded epizootics: whether it be a function of inherent virulence or ecological and/or climactic factors that could precipitate the altered epidemiological patterns observed.

Keywords

West Nile virus; Flavivirus; Horses; Equids

1. Introduction

West Nile virus (WNV) is one of more than seventy viruses of the family *Flaviviridae* of the genus *Flavivirus* that is comprised of viruses that form enveloped spherical shaped virions encompassing a single-stranded RNA molecule of positive polarity that is approximately 11-kb in length (Harris et al., 2006) (Fig. 1a and b). The genomic RNA of flaviviruses encodes three structural proteins (C-prM-E) at the 5' third of the genome and seven nonstructural proteins (NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5) in the remaining two-thirds of the genome. These are flanked by 5' and 3' untranslated regions involved in transcription and

*Corresponding author. Tel.: +493835171163. martin.groschup@fli.bund.de (M.H. Groschup).

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translation (Heinz et al., 2000). The viral structural proteins are involved in RNA encapsidation [Capsid (C)] and as scaffolding and physical structures for interactions that facilitate receptor-mediated endocytosis and envelope fusion [pre-membrane (prM/M) and envelope (E) proteins]. All seven of the nonstructural proteins have been identified to be involved in different functions associated with RNA synthesis such as RNA dependent RNA polymerase and methyltransferase (NS5), as well as helicase and protease (NS3) functions. Both structural and nonstructural proteins are translated as one large polyprotein that is cleaved into the respective viral proteins by both host and viral proteases both co- and post-translationally (Chambers et al., 1990).

Although the genus is comprised predominantly of viruses transmitted between vertebrate hosts and arthropod vectors such as mosquitoes and ticks, viruses for which no vectors have been identified [no known vectors] (Billoir et al., 2000) and viruses with no identified vertebrate hosts [insect-specific flaviviruses] have been described within the genus (Cook et al., 2011). Serologically, flaviviruses have been classified into at least eight antigenic serocomplexes based on cross neutralization characteristics with antisera generated between different viruses. Viruses belonging to the Japanese encephalitis (including WNV), tick-borne encephalitis, yellow fever and dengue serocomplexes are associated with the highest incidence of disease in humans and animals (Westaway et al., 1985).

2. West Nile virus - virology and molecular biology

West Nile viruses can be designated into at least four phylogenetic groups or lineages based on sequence analyses (May et al., 2011). Lineage 1 can be further subdivided (Fig. 2) into three clades or sublineages (a–c). Clade 1a encompasses a wide geographic range of isolates made from Africa, the Middle East, India, Europe and the western hemisphere while clade 1b represents Kunjin viruses from Australasia and clade 1c is comprised of more recent viruses from India (May et al., 2011). Clade 1c has been proposed also as new WNV lineage 5 (Bondre et al., 2007). Prior to an isolation in Hungary in 2004 (Bakonyi et al., 2006), lineage 2 WNVs had been isolated exclusively from African transmission cycles (with the exception of a single isolate in 1968 from Cyprus) (McMullen et al., 2013). Lineage 2 viruses have been circulating most commonly in Sub-Saharan Africa and Madagascar (Botha et al., 2008; McMullen et al., 2013). Lineage 2 WNVs, like lineage 1 strains, have recently undergone a radiation from Africa in which these viruses have been associated with human and equine outbreaks in Hungary (Kutasi et al., 2011; Bakonyi et al., 2013), Romania (Sirbu et al., 2011), Russia (Platonov et al., 2011) and Greece (Papa, 2012) as well as enzootic/epizootic activity in Italy (Bagnarelli et al., 2011; Savini et al., 2012) and epizootic/epidemic activity in South Africa (Venter et al., 2009). Lineage 3 (Rabensburg) virus is represented by two isolates from the Czech Republic (Bakonyi et al., 2005) that replicate very poorly in vertebrate hosts and have been postulated to be potentially arthropod-specific (Aliota et al., 2012) while lineage 4 WNV is comprised of tick isolates made from the Caucas Mountains in southern Russia (Prilipov et al., 2001). There currently is no evidence that lineage 3 or 4 WNVs have the capacity to elicit disease in humans or horses.

3. Epidemiology

West Nile virus is grouped into the Japanese encephalitis serocomplex along with other viruses transmitted between vertebrate hosts and mosquitoes such as Japanese encephalitis virus (JEV), Murray Valley encephalitis virus (MVEV) and St. Louis encephalitis virus (SLEV). Viruses belonging to this antigenic serocomplex inclusive of WNV utilize avian hosts and mosquitoes of the genus *Culex* for transmission in nature; however, all of these viruses can tangentially infect humans and a number of domestic animals. Although encephalitic syndromes can present, most domestic animals and humans fail to generate a viremia sufficient to infect mosquito vectors and are thusly referred to as “dead-end” hosts as their infection does not contribute to additional cases either directly or indirectly through infection of an arthropod vector (Fig. 3). Encephalitis in equids has been described for JEV, MVEV as well as WNV; however, WNV is responsible for the vast majority of equine flaviviral encephalitis infections worldwide with more than 600 confirmed cases reported in the United States in 2012 alone (United States Department of Agriculture, 2012). Viruses within the JEV serocomplex have a worldwide distribution with MVEV circulating in Australia, Papua New Guinea and Irian Jaya (Knox et al., 2012), JEV circulating from Pakistan-India east through northwestern Australia and SLEV circulating in North, Central and South America. West Nile virus has a much more extensive geographic range having been identified on every continent with the exception of Antarctica. Following its initial isolation from a febrile woman in the West Nile District of Uganda in 1937 (Smithburn et al., 1940), enzootic WNV transmission foci have been described in South Africa, East Africa, the Middle East, Pakistan and Australia (Kunjin). Subsequently, periodic epidemics/epizootics of human and equine disease were described in Egypt (1950s), South Africa (1970s), Israel (1950s); however, an observed increase in the frequency and distribution of these episodic events became evident in the mid 1990s. Incursions of WNV into Europe likely through bird migration directly from North Africa and East Africa through the Middle East migratory routes have been supported by viral sequence analyses from these respective geographic locations (Charrel et al., 2003). Human encephalitis epidemics in 1996 in Bucharest, Romania (Tsai et al., 1998) and 1999 in Volgograd, Russia (Platonov, 2001) as well as Israel in 1998–2000 preceded the first report of WNV in North America in New York City in 1999 in which avian deaths were associated with human encephalitis cases (Lanciotti et al., 1999) with a very similar genotype virus. Serological evidence indicated that within three years of first being identified in North America, WNV had spread to the islands of the Caribbean, Central America (Hobson-Peters et al., 2011) and portions of South America, and active transmission was documented westward across the entire North American continent and north into Canada. Despite the fact that serological evidence of WNV transmission in Latin America has existed for many years (Komar and Clark, 2006), there has been a surprisingly low incidence of equine disease in Mexico, Central America, the Caribbean islands and South America as compared to the United States and Canada. Interestingly, several cases of equine encephalitis were reported in a more temperate region of Argentina in 2006 (Morales et al., 2006), indicating the potential that climactic factors could restrict disease presentation in humans and equids via differences in the force of transmission or the potential modulating effects of host diversity (Ezenwa et al., 2006; Swaddle and Calos, 2008) in different biomes or the presence of immune responses against

heterologous flaviviruses that could afford protection against disease presentation. Sequence analyses of the few isolates of WNV that have been made in Mexico (Beasley et al., 2004) not adjacent to the U.S.–Mexican border as well as the Argentinean isolates from encephalitic horses indicate that the effects of genetic drift and the introduction of lesser virulent genotypes (Morales et al., 2006; Brault et al., 2011; Langevin et al., 2011) could play a role in reduced virulence potential.

4. Epidemiology in horses

Although WNV had been associated with sporadic epidemics in Egypt since the 1950s, epizootics in equids were not reported. However, in 1959 sporadic disease associations in equids were made, high seroprevalence rates were found and isolation of WNV from diseased horses were reported (Schmidt and Elmansoury, 1963). Fig. 4 consists of a map depicting temporal and geographic locations of WNV equine outbreaks. In France, a WNV outbreak occurred in 1962 in the Camargue region with several wild and domestic horses affected (Murgue et al., 2001b). Other cases were reported in 1965 in this region as well. In the 1990s an increase in magnitude of equine WNV disease cases was observed. In Morocco, over 90 equids were affected in an outbreak in 1996 (Murgue et al., 2001a). Two years later, WNV infection in 14 horses in Tuscany were reported (Hubalek and Halouzka, 1999; Cantile et al., 2001). In the same year, 18 horses with encephalomyelitis tested positive for WNV neutralizing antibodies in Israel with a concurrent isolation of WNV from a stork (Murgue et al., 2001a). In 1999, WNV first reached the Western hemisphere in North America and has subsequently spread throughout the North American continent and into South America. Each year equine infections have been detected with a peak of 15,257 cases in the U.S. reported during 2002 (APHIS, 2012). In 2000 and 2003, WNV infections in horses were once again reported from southern France (76 equine cases in 2000) (Murgue et al., 2001b; Mailles et al., 2003) and 2000 in Israel (131 equine cases) (Steinman et al., 2002).

Clearly, the incidence of disease in equids has increased significantly since the mid 1990s concomitant with the radiation of lineage 1a viruses. In fact, comparison of North American with European equine exposures demonstrate similarly low attack rates of approximately 5% (Nielsen et al., 2008). The effects on transmission due to environmental factors could play a significant role in the etiology of equine outbreaks due to the requirements for high infection rates for disease to be observed with various WNV lineage viruses. Equine WNV encephalitis cases increased markedly following the initial reports of diseased horses in New York State in 1999 as the virus moved westward (United States Department of Agriculture, 2012). The increase in reported equine cases that peaked in 2002 was associated with movement of the virus into areas in which *Culex tarsalis* mosquitoes served as efficient vectors for transmission (Goddard et al., 2002). Licensure of a killed WNV vaccine in late 2002 (Ng et al., 2003) resulted in a dramatic reduction in the subsequent number of equine encephalitis cases reported in 2003 despite the large increase in human cases that year (United States Department of Agriculture, 2012), indicating the efficacy of equine vaccination. In some instances in Europe, equine cases have been observed without substantial human disease being reported, such as in Tuscany, Italy (1998) (Autorino et al., 2002) and Camargue (Panthier et al., 1966), France (2000); however, in North America

equine and human cases have demonstrated similar incidence patterns, both before and after widespread vaccination of horses (Ward and Scheurmann, 2008). Since 2010, WNV infections among horses repeatedly were reported in the Mediterranean basin and other European countries, namely in Bulgaria, Croatia, Greece, Italy, Macedonia, Morocco, Portugal, Romania and Spain (Barros et al., 2011; Garcia-Bocanegra et al., 2011; OIE, 2012). The aforementioned WNV equine epizootics before 2007 were associated with lineage 1 viruses. After that year, lineage 2 viruses, which were previously considered to be less pathogenic than lineage 1 viruses, have been identified as etiological agents of severe WNV outbreaks in horses in South Africa and Hungary (Venter et al., 2009; Kutasi et al., 2011). Lineage 2 WNVs have also been associated with an ever increasing incidence of equine encephalitis in both areas associated with historical transmission such as South Africa (Venter et al., 2009; Venter and Swanepoel, 2010) as well as Europe (Kutasi et al., 2011). Once believed to be of low pathogenic potential due to high seroprevalence rates in equids in South Africa with minimal disease presentation (Guthrie et al., 2003), lineage 2 WNVs are being reevaluated as an emerging zoonotic disease threat for humans and livestock. In 2011 an equine encephalitis outbreak was observed in New South Wales, Australia in which Kunjin virus (WNV lineage 1b) was isolated and implicated as the etiologic agent in the first reported equine encephalitis outbreak attributed to this WNV genotype (Tee et al., 2012). In contrast to many lineage 1b viruses previously characterized, sequence analyses of this lineage 1b equine isolate demonstrated the virus to have a glycosylated envelope protein, and experimental inoculations of mice demonstrated the virus to have an intermediate virulence between that of lineage 1a and other lineage 1b viruses (Frost et al., 2012). To date, no specific genetic determinants have been associated with altered pathogenesis in horses despite virulence determinants having previously been associated with lineage 1 virulence differences in birds (Brault et al., 2007; Murata et al., 2010) and mice (Beasley et al., 2002, 2005).

5. Transmission of WNV

While WNV is transmitted and maintained in an enzootic mosquito-bird cycle, equids can be infected by bridge vectors and develop subclinical infection or neurological disease. Mosquito species that are believed to most likely transmit WNV to equines are *Culex pipiens* and *Culex tarsalis* in North America and *Culex pipiens* complex mosquitoes in Europe (Nielsen et al., 2008; Sebastian et al., 2008; Munoz et al., 2012).

Like humans, horses are considered to be dead-end hosts in that they develop only short and low magnitude viremia levels. In horses exposed to infected mosquito bites, Bunning et al. demonstrated a maximum serum viremia titer of 10^3 pfu/ml serum with the viremia lasting up to six days and numerous subsequent studies with experimental infection of horses have corroborated this concept (Bowen, unpublished). Such a low serum viremia coupled with the small volume of a blood meal imbibed by a mosquito (approximately one microliter) would make it unlikely that the midgut epithelia of a mosquito would be exposed to a single infectious unit of virus. Vector competency studies have confirmed that these serum viremias are not conducive to mosquito infection from WNV infected horses (Turell et al., 2008). Only one horse of 12 infected in the Bunning study became clinically ill with severe encephalomyelitis and relatively high titers of virus were observed in tissues ($10^{4.0}$ – $10^{6.8}$

pfu/tissue; Bunning et al., 2002). Other challenge studies have demonstrated maximum viremia levels of 1530 pfu/ml or lower which lasted 1–4 days, both with lineage 1 and 2 WNVs. Seroconversion occurred after 7 days post-infection with neutralizing titers of 1:320 (Minke et al., 2004; Seino et al., 2007; Minke et al., 2011).

6. Clinical symptoms in horses

Clinical signs resulting from infection of horses with WNV have been described from naturally occurring cases and experimental infections. Most horses seroconvert without clinical disease after exposure to WNV, similar to what has been described in humans. In approximately 8% of exposed naive horses, severe WNV disease with neurological symptoms develop (Gardner et al., 2007). Affected horses develop encephalomyelitis with ataxia as the predominant clinical presentation. Similar common symptoms include weakness of the limbs, recumbency and muscle fasciculation. Fewer horses present with fever, cranial nerve deficits such as a drooping lip and/or facial or muscle fasciculation; fever is inevitably observed during the course of experimental infection, but typically occurs before obvious neurologic signs. Amongst fever, also depression, anorexia, colic or lameness can be one of the first recognized signs in diseased animals (Long, 2006). Hyperesthesia, teeth grinding, muscular tremor, photophobia and blindness have been observed in a small proportion of clinical cases (Murgue et al., 2001b; Ostlund et al., 2001; Abutarbush et al., 2004; Garcia-Bocanegra et al., 2011). Abnormal behavior such as depression, somnolence, disorientation, hyperexcitability and aggressive behavior as well as changes in personality have also been associated with WN infection in equids (Ostlund et al., 2001; Porter et al., 2003; Abutarbush et al., 2004; Long, 2006; Garcia-Bocanegra et al., 2011). Excellent illustrations of the clinical signs of WN disease in horses are given by the following video: <http://www.youtube.com/watch?v=BJtVP5Bd5bs>. The fatality rate in diseased animals is high in horses and ranges from 22% to 44% (Murgue et al., 2001a; Ostlund et al., 2001; Schuler et al., 2004; Garcia-Bocanegra et al., 2011). In the 2000 French equine epizootic, fever as well as paresis and paralysis in addition to ataxia were frequent clinical signs (Murgue et al., 2001b). Fever was also common in hospitalized horses from Florida. (Porter et al., 2003). In a report from Italy, paresis of limbs, progressing to tetraplegia and recumbency predominated (Cantile et al., 2001). Disease presentation has been observed to be very similar between WNV lineage 1 and lineage 2 WNV infected equids (Venter et al., 2009; Kutasi et al., 2011), however, controlled experimental infections of equids have not been conducted in order to specifically address potential pathogenic differences between viruses of the two lineages.

A variety of horse breeds as well as a wide age distribution have been observed to present with WNV disease; however, the case fatality rate has been noted to be higher in older horses (Schuler et al., 2004). In the western U.S., females were 2.9 times more likely to succumb to infection as males (Salazar et al., 2004). Duration of illness in recovering horses ranges from two to seven days in mildly affected individuals and in excess of 20 days to several months for regeneration to their original condition of more severe disease cases (Trock et al., 2001; Venter et al., 2009). Up to 20% of diseased horses show residual sequelae such as weight loss, lethargy, ataxia or cranial nerve deficits (Salazar et al., 2004).

Similar to the high proportion of subclinical disease in natural infections, WNV challenge experiments have often failed to provoke severe clinical symptoms, sometimes with the exception of mild muscle fasciculation (Minke et al., 2004; Siger et al., 2004; Castillo-Olivares et al., 2011). Bunning et al. (2002) observed fever and severe neurological signs in one of the challenged individuals, amounting to an attack rate of approximately 8%. Only after intrathecal challenge with WN lineage 1 virus did horses develop moderate to severe neurological signs, with 50% developing fever and all requiring euthanasia (Seino et al., 2007). Similar experiments performed by intrathecal inoculation with a lineage 2 WNV lead to hyperthermia, muscle fasciculation and tremor, abnormal mentation, gait deficiency, anorexia and lip twitching as well as head shaking with an associated mortality rate of 30% (Minke et al., 2011).

Innate immune response is key to resist WNV infection (Samuel and Diamond, 2006). For mice it was shown that the interferon system is an important factor for this, as mice lacking Interferon type I receptors are highly susceptible to WNV infection (Samuel and Diamond, 2005). Moreover, there are hints that different WNV genotypes have differences in their ability to interfere in interferon signaling (Keller et al., 2006). OAS genes are induced by interferon. OAS proteins are antiviral proteins that indirectly activate Ribonuclease L, which can degrade viral RNA. Host genetic studies have indicated that polymorphisms in equine OAS 1 gene contribute to WNV susceptibility in horses. These polymorphisms were located in the interferon-inducible promoter (Rios et al., 2010). There are further studies needed to evaluate if there are more factors that modulate innate response to WNV in equines and contribute to susceptibility and resistance.

7. Pathology in horses

Pathologic alterations and immunohistochemical findings in natural WNV infected horses from Italy and the United States, respectively, were described by Cantile et al. (2001). WNV infection in horses is proposed to be limited to the CNS. In some cases, gross macroscopic changes such as petechiae in the rhombencephalon and spinal cord have been observed. Microscopically, mild to severe polioencephalomyelitis particular of lower brain stem and ventral horns of the thoracolumbar spinal cord have been detected. Interestingly, more widespread tissue distribution of U.S. horses has been observed than that noted in the Italian horses with additional involvement of basal nuclei, thalamus and midbrain. Perivascular infiltrates, glial nodules, neutrophils, hemorrhages and occasionally neuronophagia are common changes, and the cerebral cortex is spared. The quantity of WNV antigen detected by immunohistochemistry is generally low in relation to pathologic alterations, so that an immunopathologic process in equine WNV-induced disease has been considered. Due to the paucity of detected WNV antigen in endothelial cells, WNV neuroinvasion could occur through the olfactory neurons. Additionally, perivascular cuffing of lymphocytes within the perineuronal area of dorsal ganglia and moderate to severe hemorrhage in the gray matter, white matter and meninges of cervical, thoracic and lumbar segments of spinal cord were noted in Kentucky in 2002 (Sebastian et al., 2008).

Results from intrathecal challenge demonstrate corresponding histopathologic alterations as seen in natural infections (Seino et al., 2007). The same has applied to experimental

infection of equines with WNV lineage 2 viruses. No gross lesions were observed, but microscopically very mild to moderate to severe encephalitis with alterations especially in brainstem and spinal cord have been reported (Minke et al., 2011).

8. Diagnosis

There are no pathognomonic signs of WN in horses, and depending on geographic location and season, a number of other pathogens induce similar or identical clinical disease, including New World equine alphaviruses, equine herpes virus 1 and rabies virus in the U.S. and rabies, Borna and equine herpes virus 1 in the E.U. Acute WNV infections in equines can be diagnosed by the detection of the virus genome by real-time RT-PCR (syn. qRT-PCR) or by the demonstration of IgM antibodies by ELISA. A variety of well-functioning qRT-PCR methods have been published to date. Primer combinations should be selected carefully as not all work for WNV strains of both lineages (Eiden et al., 2010). The major difficulty with the qRT-PCR is the phenomenon that WNV infections in horses coincide only with a rather short viremic phase (4–6 days; Figs. 5 and 6) that is cleared by the onset of clinical disease (Bunning et al., 2002; Siger et al., 2004) so that only positive results are meaningful in a diagnostic sense. Diagnosis of WN in horses is therefore most commonly achieved by demonstrating anti-viral IgM at the time of clinical disease or by demonstration of viral antigens or RNA in nervous tissue collected post-mortem.

ELISAs are usually used for the demonstration of WNV-specific (IgM and IgG) antibodies and the presence of a high level of IgM antibodies is the best serological indication for a recent infection, as in horses they were shown to exist less than three months (Castillo-Olivares and Wood, 2004) in contrast to humans in which IgM titers may persist longer (Roehrig et al., 2003; Papa et al., 2011). IgM antibodies were also detected infrequently in horses that were vaccinated (Jonquiere et al., 2007). Many horses have IgG antibodies due to vaccination or as a consequence of an earlier subclinical WNV infection. Moreover, antibodies to a broad range of cross-reactive flaviviruses (TBEV, SLEV, etc.) can also be a cross-reactive with the antigens in many of the currently available ELISAs. Serological diagnosis of WNV infection in North America is confounded by the cross-reactivity of antibodies elicited against other flaviviruses, such as SLEV and dengue (Ledermann et al., 2011) and TBEV in Europe (Ziegler et al., 2012; Rushton et al., 2013). In order to demonstrate specific WNV antibodies sera must be run in comparative neutralization tests with the relevant viruses. A compilation of the ELISAs for the detection of WNV antibodies in horse serum that are available in North America and Europe is given in Table 1.

9. Prophylaxis and treatment

All sensible efforts should be afforded in order to minimize exposure of equids to mosquito bites; however, complete shielding of animals housed outdoors from mosquito bites, and coincidental WNV exposure, is virtually impossible. As such, minimizing outdoor activities during crepuscular time periods and using insecticide repellents has been advised.

The most efficient prevention strategy for WNV infections in horses is certainly vaccinating them with licensed vaccines (Table 2). The incursion of WNV into North America provided a potent stimulus for development of equine vaccines. The first vaccine licensed in the U.S.

was a conventionally inactivated and adjuvanted virus preparation (WEST NILE-INNOVATOR[®], Pfizer). Subsequently, two recombinant vaccines became available, one based on a canarypox vector (RECOMBITEK[®] – Equine rWNV vaccine, Merial) and the other a live-attenuated WNV-yellow fever virus chimera (PreveNile[™], Intervet). An inactivated formulation of the live-attenuated yellow fever chimeric virus (Equi-Nile[™]; Merck) has become subsequently available in the U.S. following the recall of the former. An additional inactivated WNV vaccine, Vetera[®] WNV vaccine (Boehringer Ingelheim), based on a 2005 WNV isolate, has been licensed in the U.S. In the E.U. an inactivated vaccine (Eqip[®] WNV (previously Duvaxyn[®] WNV), Pfizer) was licensed in 2009 and the canarypox vector vaccine (Proteq[™] WestNile, Merial) in 2011. Each of these vaccines is based on the New York 99 isolate of WNV, with the exception of Vetera[®], but all are predicted to protect against temporal and geographic WNVs in North America as well as alternative WNV lineages. In fact, the canarypox vectored vaccine has demonstrated protection in horses from a lineage 2 WNV challenge (Minke et al., 2011). Each of these vaccines has been widely deployed in the U.S.

There is currently no possibility for a therapeutic intervention in the case of a WNV infection other than a symptomatic treatment. These measures include electrolyte infusions against dehydration, anti-inflammatory treatment and any means of minimizing/avoiding excitation of the animals (e.g. ear plugs to reduce acoustic stimuli, light reduction, etc.) as well as extensive padding of the stall and extremities (<http://www.youtube.com/watch?v=BJtVP5Bd5bs>). Attempts can be made with parenterally administered conditionally licensed (in the U.S.) WNV antibodies, although efficacy studies are lacking (Kutzler et al., 2008). Furthermore ribavirin and interferon treatment can be attempted, but no efficacy studies in horses have been conducted (Robinson and Sprayberry, 2009).

10. Regulations and control measures

According to the OIE Terrestrial Animal Health Code (Chapter 8.16. West Nile fever) the following criteria define the occurrence of WN fever (WNF) in equids:

- WNV has been isolated from an *animal* that shows signs consistent with WNF; or
- viral antigen or viral ribonucleic acid (RNA) specific to WNV has been identified in samples from one or more *animals* that show clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected *outbreak* of WNF; or
- antibodies to WNV have been identified in an unvaccinated *animal* that shows clinical signs consistent with WNF, or that is epidemiologically linked to a confirmed or suspected *outbreak* of WNF.

The foundation for prevention of WNV infection in horses is vaccination, and, as described above, several efficacious vaccines are readily available. Control efforts center around mosquito abatement and repellents, although implementation of these strategies is difficult to achieve in many situations. Due to the low level viremia attained and apparent lack of viral shedding, an infected horse appears to pose no direct risk to other animals, including humans, except for the obvious concerns in performing a necropsy and being exposed to

infective nervous tissue by a veterinarian. Therefore, apart from isolating the affected animal mainly for animal welfare reasons, no particular control measures apply. For the same reasons there are no trade restrictions for the importation of equines coming from WNF infected countries or zones although WNV infection in horses is a reportable disease at the federal level and in most states, although quarantines are not generally applied.

11. Open questions

There are still many open questions concerning the WNV infection of equines. More research is required to address particularly the following issues:

- What are further molecular modulators triggering the outcome of the infection in horses (subclinical versus clinical/fatal)?
- What factors (ecological, veterinary and virological) could potentiate differences in the disease progression observed in Europe versus North America?
- Do differences in equine virulence exist between lineage 1 and lineage 2 West Nile viral strains?

12. Conclusions

Prior to the 1990s, WNV had been associated with low magnitude equine epizootics in Africa, the Middle East and Europe; however, since the mid-90s, WNV has been associated with larger scale equine epizootics in new geographic areas for which specific virological, ecological, climatic and/or demographic etiologies have not been associated. Newly recognized genotypes of WNV have been increasingly associated with pathogenic potential such as lineage 2 and lineage 1b (Kunjin) viruses. Equids become infected through the bite of enzootic or bridge vectors resulting predominantly in subclinical infection; however, the mortality rate of equines that present with clinical disease is high. Due to short and low magnitude viremia equids are considered “dead-end” hosts and pose no immanent health risk to other animals or humans. The most effective strategy for prevention of equine disease is proper vaccination. A number of efficacious equine vaccines have been licensed in the U.S. and E.U. including inactivated WN viral vaccines, a canarypox virus vectored recombinant and live/killed recombinant yellow fever (17D) chimeric vaccines.

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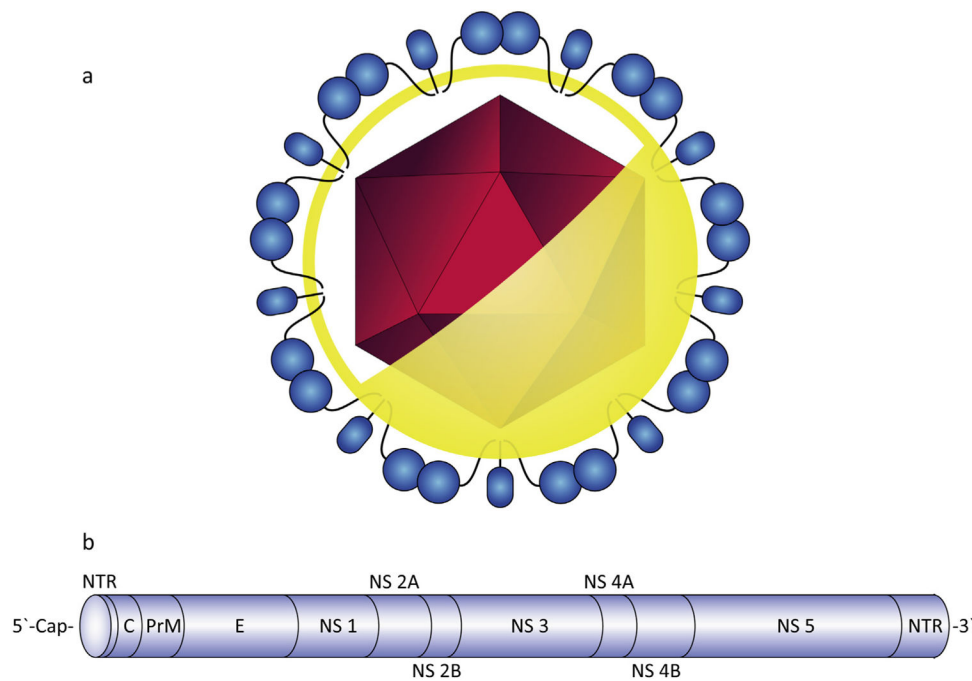
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetmic.2013.08.013>.

**Fig. 1.**

Structure of West Nile. (a) The WNV virion is composed of an icosahedral core formed by positively charged capsid (C) proteins of 12 kDa molecular mass (red). This core is enveloped by a host-cell derived membrane in which the major envelope protein E (53 kDa, blue dimers) and the membrane protein M (8 kDa, blue capsules) are integrated. C-proteins itself enclose an RNA genome encoding for a single open reading frame (ORF) of 10.302 nucleotides and non-coding regions of 96 and 631 nucleotides at the 5' and 3' ends, respectively. (b) The WNV genome encodes a polyprotein that is post-translationally processed by viral and cellular proteases into five non-structural proteins (NS1, NS2A/NS2B, NS3, NS4A/NS4B and NS5) and into three structural proteins (C, prM/M and E). E protein and prM/M protein (18–20 kDa) virus. (For interpretation of the references to color in Figs. 1 and 2 legends, the reader is referred to the web version of this article.)

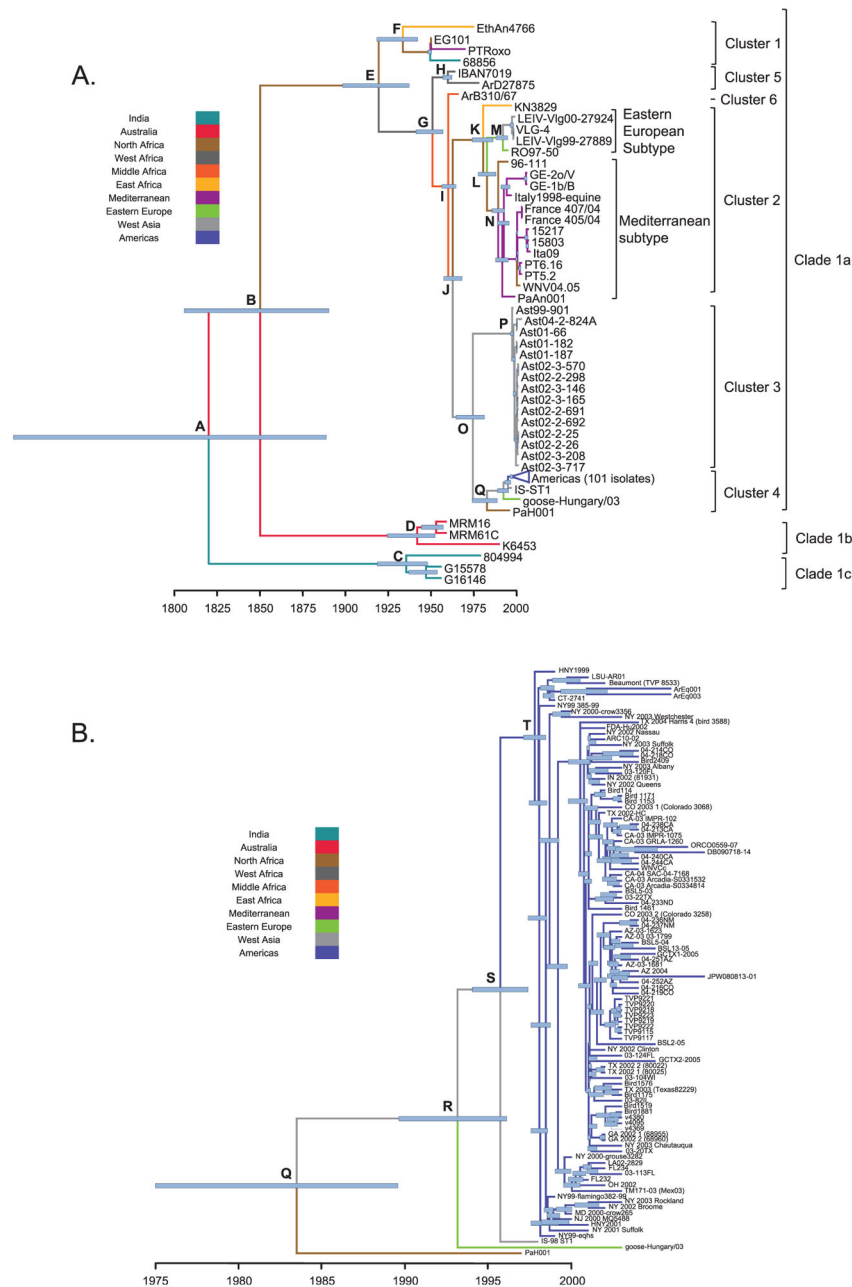
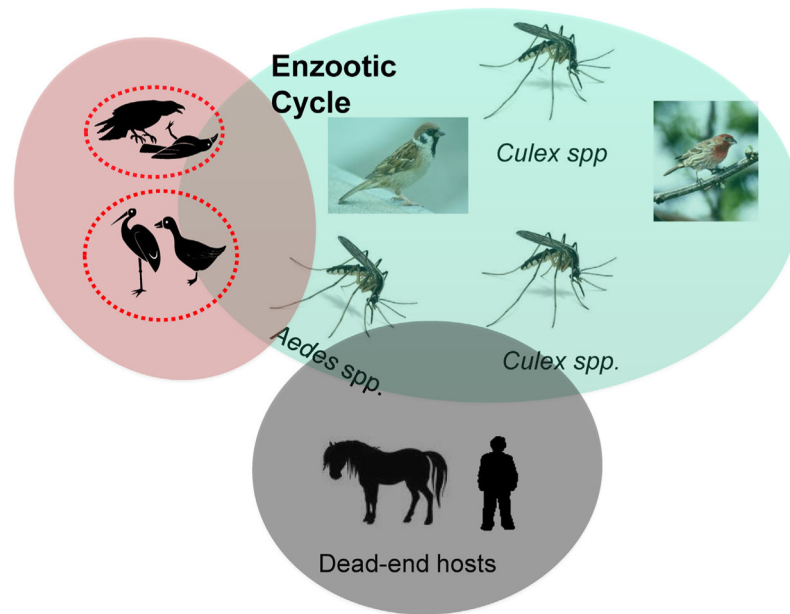


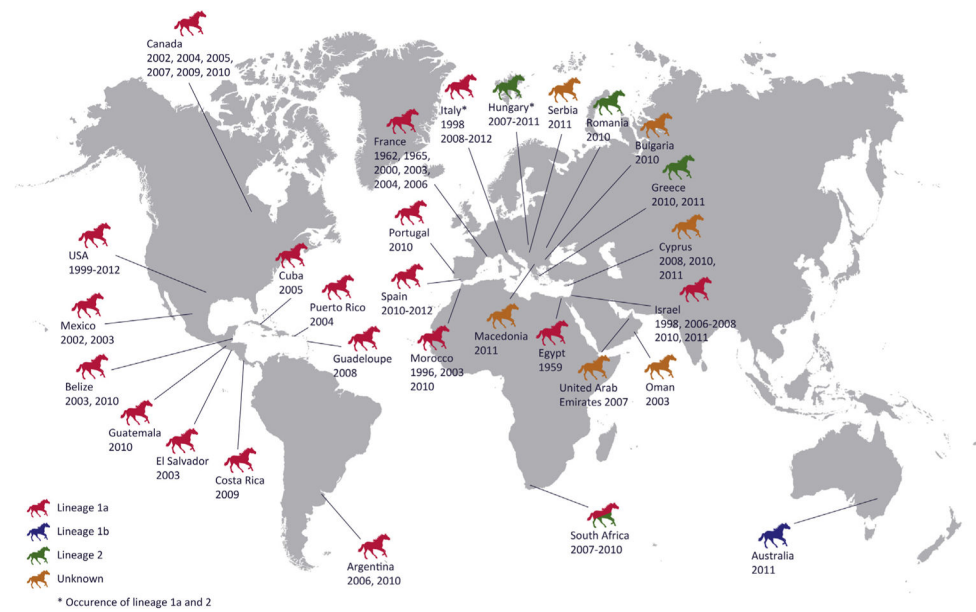
Fig. 2.

This figure is reused from the *Journal of Virology*, 2011, volume 85 (6), pp. 2964–2974, DOI 10.1128/jvi.01963-10 (May et al., 2011), reproduced/amended with permission from American Society for Microbiology and kindly provided by Alan Maximum clade credibility (MCC) tree of WNV genomes constructed using BEAST version 1.5.3 software. Colors of branches indicate geographic locations per the color key. Branch lengths correspond to lengths of time, as measured by the scale bar, and the 95% HPD range of divergence dates is shown as a bar. The letters at the nodes correspond to data in Table 1.

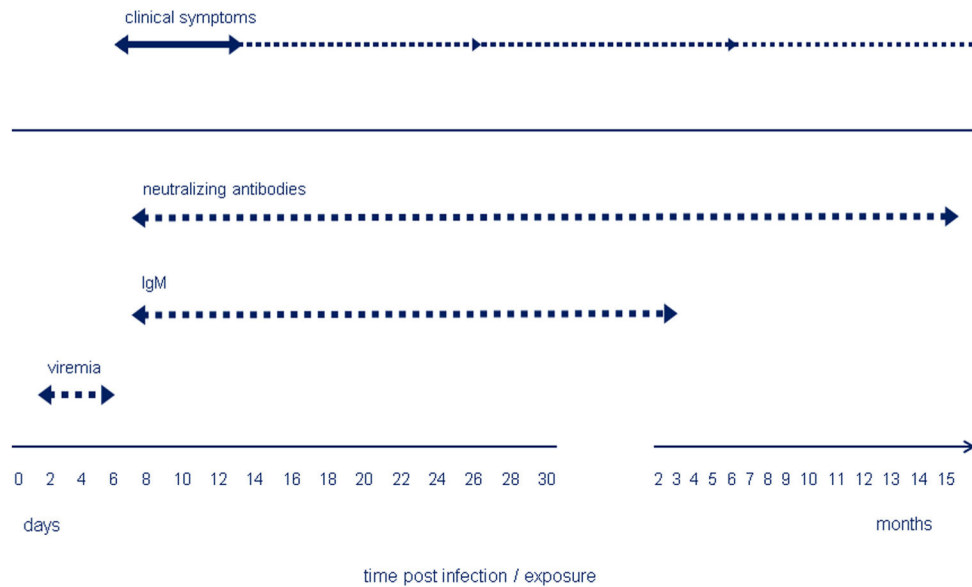
(A) All isolates (with the branch of isolates from the Americas collapsed). (B) Cluster 4 isolates only (May et al., 2011). Barrett.

**Fig. 3.**

Bird/mosquito transmission cycle of WNV is transmitted in an enzootic passeriform bird–mosquito (*Culex* spp. mosquitoes) cycle (green shading). Bridge vectors (such as *Aedes* spp.) can transmit the virus to humans and equines (gray shading) that are designated as “dead-end” hosts in that they can become infected but are not capable of serving as a source of infection for additional mosquito vectors. Some peridomestic avian hosts (red shading and dashed ovals; such as corvids) are capable of developing high titers following infection with certain WNV strains and facilitate epidemic amplification. WNV.

**Fig. 4.**

Equine WNF cases reported worldwide officially reported to WAHID/OIE or PROMED or published in literature since 1959. Lineage color codes were assigned if at least one horse isolate of this lineage was found or if this lineage was the only published lineage occurring in this country (for example in humans, birds, mosquitoes).

**Fig. 5.**

Virus Infection and immune response in horses. WNV infected viremia is observed in the first six days post-infection and can last 1–6 days (Bunning et al., 2002; Minke et al., 2004; Seino et al., 2007). Antibodies can be detected from day 7 or 8 post-infection. Detection of IgM is possible for less than 3 months (as reviewed in Castillo-Olivares and Wood, 2004), whereas neutralizing antibodies persist for at least 15 months (Ostlund et al., 2001). Clinical symptoms are thought to occur mostly after the end of viremia (Castillo-Olivares and Wood, 2004), nevertheless the onset of symptoms in naturally acquired infections can only be estimated. Following mild infections, equines usually recover after two to seven days, but may need more than 20 days up to several months for complete reconstitution to their original condition (Trock et al., 2001; Venter et al., 2009). In some cases residual signs of the infection may even persist (Salazar et al., 2004).

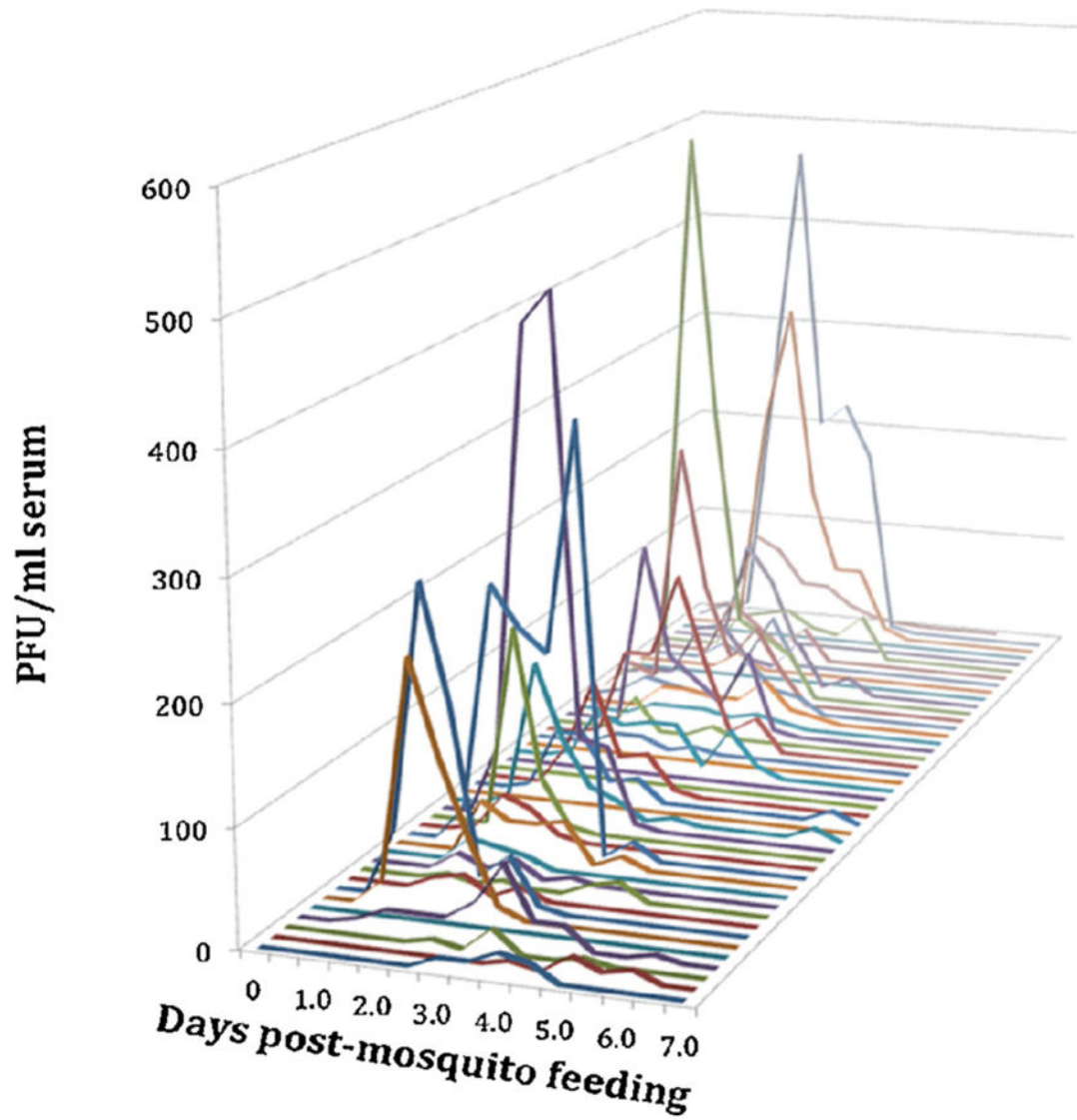


Fig. 6. Serum viremia profiles for 47 horses experimentally infected with WNV NY99 by feeding of infected mosquitoes. No horse had a demonstrable viremia beyond 6.5 days post-feeding.

Table 1

Commercially available ELISAs for the detection of WNV antibodies in horse serum.

Brand name(s)	Principle	Detected antibodies	Antigen WNV lineage	Comments
ID Screen® West Nile Competition ELISA	Competitive ELISA	IgG, IgM	E protein Lineage 1	Cross-reactive with other flaviviruses
ID Screen® West Nile IgM Capture ELISA	Indirect capture ELISA	IgM	E protein Lineage 1	
IDEXX IgM WNV Ab Test	Indirect capture ELISA	IgM		
Prionics/Ingenasa INGEZIM WEST NILE Compac	Competitive ELISA	IgG, IgM	E protein Lineage 1	

Table 2

WNV vaccines for use in horses.

Brand name(s)	Approved in	Formulation	WNV Lineage	Application	Booster	References
Duvaxyn® WNV, Fort Dodge (→ now Equip® WNV, Pfizer in the E.U.; WEST NILE-INNOVATOR®, Pfizer in U.S.)	U.S., E.U.	Formalin inactivated whole WNV VM-2	1	1 ml i.m.	After 3–5 weeks, then annually	Ng et al. (2003), Seino et al. (2007)
RECOMBITEK® – Equine rWNV vaccine, Merial, U.S., approved in the E.U. as Proteq West Nile™	U.S., E.U.	Recombinant live canarypox virus ALVAC which co-expresses WNV prM and E proteins of a NY99 isolate	1	1 ml i.m.	After 4–6 weeks, then annually	Minke et al. (2004), Siger et al. (2004, 2006), Seino et al. (2007), El Garch et al. (2008), Minke et al. (2011)
Veterra® WNV vaccine, Boehringer Ingelheim	U.S.	Inactivated vaccine	1	1 ml i.m.	After 3–4 weeks, then annually	
Equi-Nile™, MERCK	U.S.	Inactivated vaccine, WNV prM/E proteins in YF17D backbone	1	1 ml i.m.	After 3–4 weeks, then annually	
PreveNile™, Intervet (recalled 2010)	U.S.	Live flavivirus chimera, WNV prM/E proteins in YF17D backbone	1	1 ml i.m.	Annually	Seino et al. (2007)

U.S., United States; E.U., European Union.